J.Vet. Malaysia (2003)15 (1 & 2): 15 - 19

PHENOTYPIC CHARACTERISATION OF *PASTEURELLA MULTOCIDA* OBTAINED FROM POULTRY IN IRAN

A.R. Jabbari[1], A.A. A. Saharee[2] and F. Esmaily[1]

[1]Biotechnology Department, Razi Vaccine and Serum Research Institute, Karadj, Iran
[2]Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

SUMMARY

Phenotypic patterns of twenty five isolates of *P.multocida* strains isolated from northern provinces of Iran were determined by using different carbohydrates from hexoses, pentoses, disaccharides and polyhydric alcohols. The strains showed a high homogeneity. All strains were able to ferment sorbitol, manitol, galactose, dextrose, fructose, glucose, mannose and sucrose. However none of them could produce acid from dulcitol, inositol, arabinose, salicine, rhafinose and inoline. According to this pattern all of isolates belonged to subspecies multocida. The *in-vitro* sensitivity of isolates against 13 chemotherapeutic agents was determined by the paper disc method. Among the antibiotics tested, chloramphenicol, combination of sulfamethoxazine and trimethoprim and nitrofurantoin were found to be the most effective (100%) followed by tetracycline (96%), penicillin (88%), and gentamycine (76%). Strains showed 100% resistance to lincomycin and bacitracin followed by 84% and 68% resistance to furazolidon and colistin respectively. All *P.multocida* isolates identified as to capsular type A.

Key words: *Pasteurella multocida*, poultry, phenotype

INTRODUCTION

Pasteurella multocida a gram negative bacteria is the cause of fowl cholera and other important diseases in a variety of animals. Fowl cholera has been recognised as an important disease in domestic poultry for more than 200 years as it has caused major economic losses in the poultry industry (Morishita *et al.*, 1996a).

Separation of isolates of *P. multocida* into subgroups or biotypes based upon variations in biochemical characteristics has been reported (Weaver and Hollis, 1980; Blackall *et al.*, 1995; Fegan *et al.*, 1995). This sub grouping was based mostly upon reaction patterns observed with acid production from certain pentoses (like xylose and arabinose), disaccharides (like maltose and trehalose) and polyhydric alcohols (like sorbitol, manitol and dulcitol).

Higher incidences of certain fermentation reaction for isolates from a particular animal species have been reported. The aims of this study were to determine the biochemical characteristics of avian *Pasteurella multcida* isolates from poultry and their (biotype), and to determine the antibiotic sensitivity patterns of the isolates against different therapeutic agents and identify the capsular group of the isolates.

MATERIALS AND METHODS

Isolation and identification

Swab samples were taken from heart blood and liver of suspected freshly dead poultry submitted to veterinary clinics in order to diagnose the disease. The isolates were identified on the basis of colony morphology, growth on blood agar without haemolysis but not on MacConkey agar, Gram negative coccobacillus, oxidase and catalase positive, presence of b-galactosidase activity, indole positive, sulfite, citrate and motility negative and absence of urease activity (Quinn *et al.*, 1994). Carbohydrate fermentation test was conducted to identify the ability of the isolates to catalyse arabinose, dextrin, dulcitol, galactose, glycerol, inositol, inulin, lactose, maltose, manitol, raffinose, sorbitol, sucrose, trehalose, and xylose.

Differential identification and biochemical characteristics of the isolates were determined according to Fegan *et al.* (1995).

Antimicrobial Sensitivity Determination

The drug resistance of *P.multocida* isolates was determined on Mueler-Hinton's agar according to Bauer disc diffusion method (Bauer *et al.*, 1966). The antibiotics and chemotherapeutics used and their concentrations per disc were as follows: sulfamethoxazin-trimetoprim (SXT 300 + 80 *mg*), chloramphenicol (C 30 *mg*), furazolidon (FR 100 *mg*), gentamycin (GM 10 *mg*), streptomycin (S 10 *mg*), cloxacillin (CX 5 *mg*), penicillin (P 10 IU), tetracycline (T 30 *mg*), erytromycin (E 15 *mg*), Colistin (CL 10 *mg*), lincomycin (L 2 *mg*), Nitrofurantoin (FM 300 *mg*), and bacitracin (Ba 5 *mg*). The tests were repeated 3 times for each isolate. Results were analysed on the base of the inhibition zones according to the procedure described by Quinn *et al.* (1994).

Determination of the capsular antigen

P. multocida isolates were tested for group A capsule using the hyaluronidase test as described by Carter and Rundell (1975). The hyaluronidase producing culture of *Staphylococcus aureus* was streaked across a freshly

prepared dextrose starch agar plate. The *Pasteurella multocida* cultures to be tested were streaked across at right angles. Hyaluronic acid production was indicated by a reduction in size of Pasteurella growth adjacent to the *S. aureus* streak.

RESULTS

Isolates

Fourteen *P. multocida* were isolated from 68 suspected samples that were sent to Razi Vaccine and Serum Research Institute (RVSRI) from different parts of endemic area by veterinary practitioners. Eleven *P. multocida* cultures, which had been isolated during previous outbreaks, were obtained from RVSRI. Table 1 shows the origins and districts of the isolates.

Biochemical characteristics

All isolates were Gram negative rods and produced indole, oxidase, catalase, and β-galactosidase. None of the isolates were able to produce urease and use citrate as energy source. They grew on blood agar without hemolysis but not on MacConkey agar. Biochemical characterisation of the isolates to determine acid production from different carbohydrates showed that all isolates fermented sorbitol, manitol, galactose, dextrose, fructose, mannose and sucrose. However none of them fermented dulcitol, inositol, arabinose, salicin, rhaffinose, trehalose, xylose and inoline.

Table 1: Origins of twenty five *Pasteurella multocida* isolates obtained from poultry in northern part of Iran

Isolate	Origin	Province	Source
PMI032	Chicken	Mazandran	Present study
PMI033	Duck	Mazandran	Present study
PMI034	Chicken	Gilan	Present study
PMI035	Duck	Mazandran	Present study
PMI036	Geese	Mazandran	Present study
PMI037	Duck	Gilan	Present study
PMI038	Geese	Mazandran	Present study
PMI039	Duck	Mazandran	Present study
PMI040	Chicken	Mazandran	Present study
PMI041	Chicken	Mazandran	Present study
PMI042	Chicken	Mazandran	RVSRI
PMI043	Chicken	Mazandran	RVSRI
PMI044	Duck	Mazandran	RVSRI
PMI045	Duck	Mazandran	RVSRI
PMI046	Chicken	Mazandran	RVSRI
PMI047	Chicken	Mazandran	Present study
PMI022	Chicken	Mazandran	Present study
PMI023	Chicken	Gilan	Present study
PMI024	Chicken	Gilan	Present study
PMI025	Chicken	Gilan	RVSRI
PMI026	Chicken	Mazandran	RVSRI
PMI028	Chicken	Mazandran	RVSRI
PMI030	Chicken	Gilan	RVSRI
PMI031	Duck	Gilan	RVSRI
PMI020	Chicken	Mazandran	RVSRI

All isolates produced indole and none of them produced hydrogen sulphide (H_2S). They were negative in liquifaction of gelatin and production of urease. According to biochemical characteristics described by Fegan *et al.* (1995), it was revealed that all isolates were *P. multocida* and belonged to subspecies (biotype) multocida.

Antimicrobial sensitivity test

Tables 2 and 3 show the results of antimicrobial sensitivity test of the isolates against thirteen different therapeutic agents. This investigation showed that all isolates of *P. multocida* were resistant to at least 3 of the antimicrobials tested. Strain PMI032 had the widest range of resistance. It was resistant against 7 of 13 antimicrobial agents respectively. Isolates PMI034, PMI041, PM044, PMI045, and PMI023 had the most frequency of sensitivity, being sensitive to 8 of 13 therapeutic agents.

Among the antibiotics tested, chloramphenicol, combination of sulfametoxazin and trimetoprim and nitrofurantoin were found to be the most effective (100% sensitivity) followed by tetracycline (96% sensitivity), penicillin (88% sensitivity) and gentamycin (76% sensitivity).

Frequency of resistance was found most against lincomycin, bacitracin and cloxacillin (100% resistance) followed by furazolidon and colistin with 84% and 68% resistance respectively. Among 25 isolates of *P. multocida* only one strain (strain PMI037) was resistant against tetracycline. In this study, 23 isolates (92%) showed intermediate sensitivity to erythromycin.

Capsular typing

All isolates demonstrated capsular antigen group A in hyaluronidase test.

DISCUSSION

Twenty-five *P. multocida* isolates comprising 14 (eight from chicken, four from ducks, and two from geese) obtained in the present study and 11 isolates supplied by RVSRI were examined for their biochemical characteristics and antimicrobial sensitivity patterns. All isolates showed similar biochemical activities and identified as *P. multocida* subspecies multocida and capsular type A. These findings were in agreement with the previous studies (Snipes *et al.*, 1990a; Morishita *et al.*, 1996a,b; Blackall *et al.*, 1995). Snipes *et al.* (1990a) conducted a study on *P. multocida* isolates from turkey and wild life in California. They demonstrated that biochemical profile were fairly uniform among the isolates. They also observed that the majority (95%) of avian isolates belonged to subspecies multocida. Fegan *et al.*, (1995) performed an extensive study on the phenotypic characterisation of avian *P. multocida* and found that 82.5% of isolates belonged to subspecies multocida and a few were identified as subspecies galicida and septica.

It was demonstrated that acid production from maltose, trehalose and dextrin occurred more frequently with isolates

Table 2: Number and percent of sensitive, intermediate and resistant isolates of avian *P.multocida* against 13 therapeutic agents

Antimicrobial agent	Amount per disc	Sensitive isolates		Intermediate isolates		Resistant isolates	
		No	%	No	%	No	%
Cloramphenicol	30 μg	25	100	0	0	0	0
Sulfamethoxazin + Trimetoprim	300+80 μg	25	100	0	0	0	0
Furazolidon	100 μg	1	4	3	12	21	84
Gentamycin	10 μg	19	76	4	16	2	8
Erythromycin	15 μg	1	4	23	92	1	4
Streptomycin	10 μg	14	56	9	36	2	8
Penicillin	10 IU	22	88	0	0	3	12
Tetracycline	30 μg	24	96	0	0	1	4
Colistin	10 μg	0	0	8	32	17	68
Lincomycin	2 μg	0	0	0	0	25	100
Nitrofurantoin	300 μg	25	100	0	0	0	0
Bacitracin	10 μg	0	0	0	0	25	100
Cloxacillin	5 μg	0	0	0	0	25	100

Table 3: Distribution of *P. multocida* isolates according to their multiple antibacterial resistance

No of resistance	No of isolates	% of isolates	strains
Resistance to 3 agents	1	4	PMI033
Resistance to 4 agents	8	32	PMI030,PMI036,PMI040, PMI041,PMI022,PMI023,PMI034, PMI,43
Resistance to 5 agents	10	40	PMI038,PMI039,PMI044,PMI045,PMI020,PMI024, PMI026,PMI031,PMI042, PMI028
Resistance to 6 agents	5	20	PMI032,PMI037,PMI046, PMI047,PMI025
Resistance to 7 agents	1	4	PMI035

from dogs compared to isolates from other animals. In the present study, isolates that have the ability to produce acid from sorbitol, but not from arabinose, dulcitol and maltose were most frequently isolated from poultry in Iran. These findings were similar to those reported by Snipes *et al.*, (1990a) and Fegan *et al.* (1995). No clear-cut relationship has been established between clinical, epidemiology, virulence or serotyping and a pattern of acid production from substrates.

According to the results of this study, all of the *P. multocida* isolates evaluated had at least 3 antimicrobial resistance. The greatest sensitivity was shown against chloramphenicol, nitrofurantoin, Sulfamtoxazin-trimetprim combination and tetracycline. Similar sensitivity to antibiotic agents has been reported previously. Avian isolates of *P.multocida* were sensitive to tetracycline (Morishita *et al.*, 1996a; Walser and Davis, 1975), sulfonamides (Morishita *et al.*, 1996a,b; Waltman and Horne, 1993), penicillin G (Rajiini *et al.*, 1995; Morishita *et al.*, 1996a; Waltman and Horne, 1993), chloramphenicol (Rajiini *et al.*, 1995) and erythromycin (Morishira *et al.*, 1996b; Walser and Davis, 1975).

Penicillin is an antibiotic well known for its effectiveness on Gram positive organisms. Although *P. multocida* is a Gram negative bacteria, it was sensitive to this antibiotic *in vitro*, however it was not effective on the organism *in vivo* (Bain *et al.*, 1982).

Tetracycline was tested as a representative of tetracycline family (oxytetracycline, chlortetracyline and doxycycline). Among the 25 *P.multocida* isolates examined against tetracycline, only one (4%) was resistant. The tet (H) gene is known to be responsible for resistance to tetracycline. Hansen *et al,* (1996) demonstrated that the tet (H) gene is responsible for resistance among *P. multocida* isolates. They found that 25 of 31 resistant isolates tested contained the tet (H) gene. The gene was found on chromosomal DNA as well as on plasmid, suggesting that it is carried on a transposable element.

A strain may become resistant to antibiotics by two different genetic mechanisms: mutation in a chromosomal gene or infection by a plasmid. The later mechanism presents a much more serious problem, because it is more prevalent, it usually involves resistance to multiple agents, and it does not substantially decrease the growth rate or the virulence of organisms (Davis *et al.*, 1990). According to findings of the present study (Table 2), it was very interesting to note that 9 of 10 isolates that showed multiple resistance to 5 antibiotics had the same resistance pattern and originated from the same geographical area (Mazandaran). They were resistant to colistin, lincomycin,

furazolidon, bacitracin and cloxacillin. It is probable that these isolates have been infected with the same transposable carrier (R- plasmid). Further investigation is suggested for detection and identification of this genomic carrier and its origin. Although antibiotic sensitivity patterns and multiple resistance have been identified among avian pathogenic bacteria such as *E. coli*, Salmonella and Proteus in Iran (Gholamian, 1996), this is the first published data on avian *P. multocida.*

Antibiotics have been used for different purposes in the poultry farms in the endemic area as other parts of the country. According to field observations, the abuse and anarchic use of antibiotics is common among the poultry farmers in the area. They sometime use antibiotics such as lincomycin, colistin, and furazolidon for a long time to prevent enteric and pulmonary bacterial infections (veterinary practitioner, Roohani.M). Abuse of antibiotics not only can cause drug resistance but also make major problems for public health. Prolonged use of antibiotics decreases the population of sensitive bacteria, whereas, the condition becomes more susceptible for growth of the resistant (mutant) organisms.

The data from this study may serve as a guideline in selecting drugs to be used for treating poultry affected by avian pasteurellosis. In treating an acute fowl cholera outbreak, it is not logical to wait for the result of antibacterial sensitivity test. The treatment has to begin with an effective antibacterial agent till the result of antibiogram is obtained. According to the findings of the present study, Tetracyclines and sulfonamide-trimethoprim combinations are the drugs of choice for this purpose.

AKNOWLEDGMENTS

The authors are grateful to the support of the Razi Vaccine and Serum Research Institute (RVSRI). This work was supported by funds provided by the Iranian Ministry of Jahad Agriculture.

REFERENCES

Bain R.V.S., De Alwis, M. C. L., Carter, G. R. and Gupta, B. K. (1982). Haemorrhagic septicaemia. FAO Animal Production and Health Paper No. 33. FAO, Rome.

Blackall, P. J., Pahoff, J. L., Marks, D., Fegan, N. and Morrow, C. J. (1995). Characterization of *Pasteurella multocida* isolated from fowl cholera outbreaks on turkey farms. *Aust. Vet. J.* **72:** 135-138.

Bauer A. W., Kirby, W. M. N., Sherris, J. C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Path.* **36:** 493-496.

Carter, G.R and Rundell, S. W. (1975). Identification of type A strains of *Pasteurella multocida* using staphylococcal hyaluronidase. *Vet. Rec.* **96:** 343.

Davis, B. O., Dulbecco, D. R., Elsen, H. N., Glnsberg, H. S. (1990). Microbiology. 4th. Ed. J. B. Lippincott Company. pp. 220-221

Fegan, N., Blackall, P. J. and Pahoff, J. L. 1995. Phenotypic characterization of *Pasteurella multocida* isolated from Australian poultry. *Vet. Microbiol.* **47:** 281-286.

Gholamian D. A. (1996). Antibiotic sensitivity pattern among avian pathogenic bacteria in shahrekord. Thesis number 2513 submitted for a DVM degree to the Faculty of Veterinary medicine, Tehran University. pp. 189-190.

Hansen, L.M., Blanchard, P.C. and Hirsh, D.C. (1996). Distribution of tet (H) among pasteurella isolates from United States and Canada. *Antimicrob. Agents. Chemother.* **40:** 1558-60.

Morishita, T.Y., Lowestine, L.J., Hirsh, D.C. and Brooks, D.L. (1996a). *Pasteurella multocida* in raptors : prevalence and characterization. *Avian Dis.* **40:** 908-918.

Morishita, T.Y., Lowestine, L.J., Hirsh, D.C. and Brooks, D.L. (1996b). *Pasteurella multocida* in Psittacines: prevalence, pathology, and characterization of isolates. *Avian Dis.* **40:** 900-907.

Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.R. (1994). Clinical Veterinary Microbiology. Wolf Publication. pp. 95-98.

Rajini, R., Rao, S., Dhanalakshmi, K. and Sarma, B. J. R. (1995). Studies on avian pasteurellosis in Andhra Pradesh. 1989. *Indian Vet. J.* **72:** 115-118.

Snipes K.P., Hirsh, D. C., Kasten, R. W., Carpenter, T. E. Hird, D. W. and MacCapes, R. H. (1990a). Homogeneity of characteristics of *Pasteurella multocida* isolated from turkeys and wildlife in California. *Avian Dis.* **34:** 315-320.

Walser, M. M. and Davis, R. B. (1975). *In vitro* characterization of field isolants of *Pasteurella multocida* from Georgia turkeys. *Avian Dis.* **19:** 523-532.

Waltman, W. D. and Horne, A. M. (1993). Characterization of fowl cholera diagnosed in Georgia 1989-91. *Avian Dis.* **37:** 616-621.

Weaver, R.E, and Hollis, D. G. (1980). Manual of Clinical Microbiology. 3rd ed. American Society Microbiology. Washington D. C. pp. 242-262.

RINGKASAN

Corak fenotip untuk dua-puluh lima isolat strain *P. multocida* yang dipencilkan dari wilayah utara Iran telah ditentukan dengan mengguna pelbagai karbohidrat daripada heksosa, pentosa, disakarida dan alkohol polihidra. Strain tersebut menunjukkan keseragaman yang tinggi. Kesemua strain dapat menapai sorbitol, manitol, galaktosa, dekstrosa, fruktosa, glukosa, mannosa dan sukrosa. Walaubagaimanapun, tiada strain dapat menghasilkan asid daripada dulsitol, inositol, arabinosa, salisina, rafinosa dan inolina. Mengikut corak ini, kesemua isolat adalah kepunyaan subspesies, *multocida*. Kepekaan *in vitro* isolat terhadap 13 agen kimoterapeutik ditentukan melalui kaedah cakera kertas. Di antara antibiotik yang di uji, kloramfenikol, gabungan sulfametokasazin bersama trimetroprim dan nitrofurantoin didapati sangat berkesan (100%) diikuti oleh tetrasiklin (96%), penisilin (88%) dan gentamaisin (76%). Strain menunjukkan 100% kerentangan terhadap linkomaisin dan basitrasin, diikuti dengan masing-masing 84% dan 68% kerentangan terhadap furazolidon dan kolistin. Kesemua isolat *P. multocida* telah dikenalpasti sebagai tip A kapsul.